Misya Christina's Lab Notebook

June 1, 2015

Objectives:

- Perform genetic transformation of pGLO into E.Coli cells
- Practice lab skills & sterile techniques, especially for the iGEM members that have not been acquainted to Bio Lab

Results

- pGLO Transformation from yesterday did not work. It may be due to the fact that we used old plates, and therefore we need to make new ones

June 2, 2015

Objectives:

- Learn about lab preparation (autoclaving, turning on the water bath, sterilizing the hood)
- Made Plates:
  - LB
  - LB + Amp
  - LB + Amp + Ara
  - LB + Amp + Ara + Glucose
- Successfully rehydrate plasmids from the kit and transform using the iGEM protocol and dH5 α

Procedures

- Learned about lab preparation and started to learn to make new plates. Re-did the pGLO transformation with the new plates.
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Results

- pGLO Transformation did not work. The possibilities that could happen:
  - Competent cells did not work properly
  - Plates used were not good
    - We realised that instead of using 4g/100mL ratio to make LB Agar, we used 20g/250mL, which may have affected our plates.
  - Plasmids used were not viable - but this is very unlikely. In order to overcome this, we will do:
    - Transformation with the plates we made today and original pGLO
    - Transformation with a set of new plates and not original pGLO
    - Transformation with a set of new plates and the original pGLO
- We made mistake while plating: putting the antibiotic ampicillin before the flask completely cooled down. We overcame this by waiting for it to cool down and then adding the proportional amount of ampicillin.

June 3, 2015

Objectives:

- Troubleshoot the pGLO transformation
- Prepare rehydrated plasmids from the kit to successfully result in fluorescent colonies

Procedures:

- In order to troubleshoot the pGLO transformation, we did:
  - Made more plates
  - Transformation with the plates we made today and original pGLO
  - Transformation with a set of new plates and not original pGLO
  - Transformation with a set of new plates and the original pGLO

Results:

- Still no viable transformation results. Next week, Lina (our lab technician) will try to do the transformation with Christina to see if there is anything wrong procedurally.

June 4, 2015

Objectives:

- Make chloramphenicol plates with different concentrations

Procedures:

- Christina made different concentrations of chloramphenicol stock solutions and then made plates containing the chloramphenicol with the different concentrations:
  - 12.5 mg/ml Chloramphenicol Stock Solution
  - 25 mg/ml Chloramphenicol Stock Solution
  - 34 mg/ml Chloramphenicol Stock Solution
  - 50 mg/ml Chloramphenicol Stock Solution

Results:

- Encountered some difficulties while making the plates. The LB agar solidified before we finished plating, we had to reheat it and add more arabinose/chloramphenicol afterwards.
- Our instructor advised us to only make 25mg/ml and we don't need to make the rest because 25mg/ml is usually the standard concentration.

June 5, 2015

Objectives:

- Perform miniprep on the RFP and CFP amplified bacteria

Procedures:

- For this first miniprep, we call it a lab skill workshop, so everyone was involved in purifying the plasmids; we had Xiao Yue, Spencer, Zhang Zhan, So High, Reida, and Christina working on different samples. We followed the BioRad Miniprep Protocol.

Results:

Sample #	BioBrick	Plate	Well	Content	Plate Content	NA Concentration	Unit	260/280	Date
1	BBa_J04451	3755	2F	Generator: RFP	4ml LB/amp/glu RFP 10ul #1	87.6	ng/µl	2	6/5/2015
2	BBa_J04452	3755	2F	Generator: RFP	2ml LB/amp/glu RFP 10ul #2	67.8	ng/µl	1.93	6/5/2015
3	BBa_J04454	3755	2F	Generator: RFP	2ml LB/amp/glu RFP 100ul #1	31.3	ng/µl	2.01	6/5/2015
4	BBa_J04453	3755	2F	Generator: RFP	4ml LB/amp/glu RFP 100ul #2	60.6	ng/µl	1.89	6/5/2015
5	BBa_J04450	3755	2F	Generator: RFP	5ml LB/amp RFP 100ul	124.7	ng/µl	1.91	6/5/2015
9	BBa_I13600	3755	23F	Generator: CFP	4ml LB/amp 23F 200ul #1	42.5	ng/µl	1.93	6/5/2015
10	BBa_I13600	3755	23F	Generator: CFP	2ml LB/amp 23F 200ul #2	29.6	ng/µl	1.98	6/5/2015
7	BBa_J04456	3755	15J	Generator: CFP	2ml LB/amp 15J 200ul #1	53	ng/µl	1.89	6/5/2015
8	BBa_J04457	3755	15J	Generator: CFP	4ml LB/amp 15J 200ul #2	48.4	ng/µl	1.76	6/5/2015
6	BBa_J04455	3755	11J	Generator: CFP	5ml LB/amp 11J 200ul	56.4	ng/µl	1.97	6/5/2015

June 9th 2015

Objectives:

- Do a transformation with Lina in order to see if there was anything wrong with the transformation procedurally.

Procedures:

- Figured out that the problem with the transformation is that you don't need to put 250µl of the CaCl solution because the cells are already competent (and treated with CaCl). Christina proceeded with the transformation using plates Lina made and plates she made on June 2nd.

Results:

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Plate Used	Observation (2 µL +pGLO plasmid and 50µL bacteria)
LB + Ampicillin + Arabinose (By Lina)	Colonies Fluoresce
LB + Ampicillin (By Lina)	Colonies Grew
LB + Ampicillin + Arabinose (June 2nd)	Colonies Fluoresce
LB + Ampicillin (June 2nd)	Colonies Grew

June 10, 2015

Objectives:

- Grew Liquid Cultures to start making Competent Cells
- Perform a gel electrophoresis of purified plasmids and perform a gel extraction

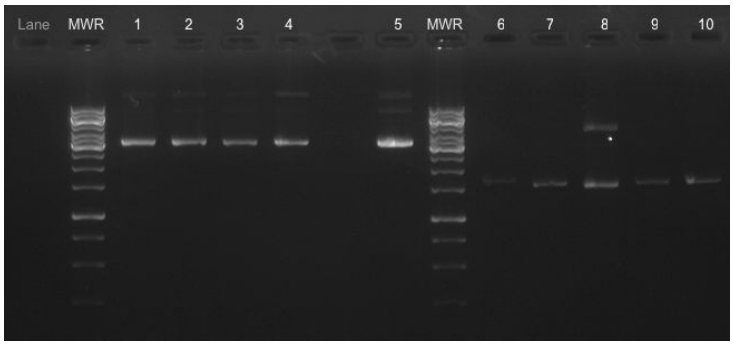
Procedures:

- Plated the cultures on an LB plate and then incubated them overnight
- Reida, Ann, and I all made agarose gel, but all at different concentration; Ann made a 1% gel, Reida .7%, Christina .4%. We used the TIANgel Midi DNA Purification Kit Protocol #1.

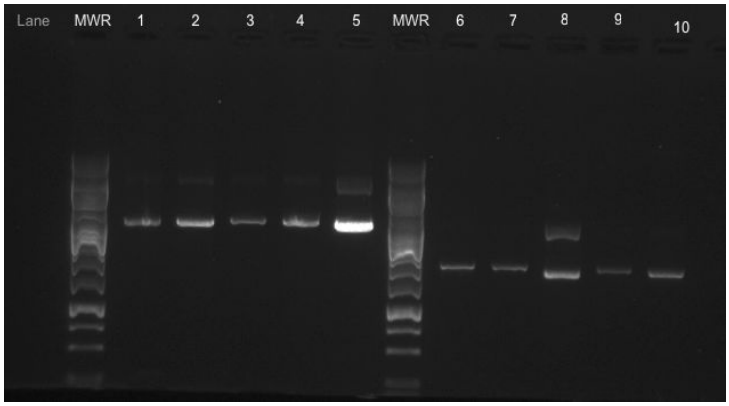
Results:

- Plates with LB cultures had healthy colonies grown

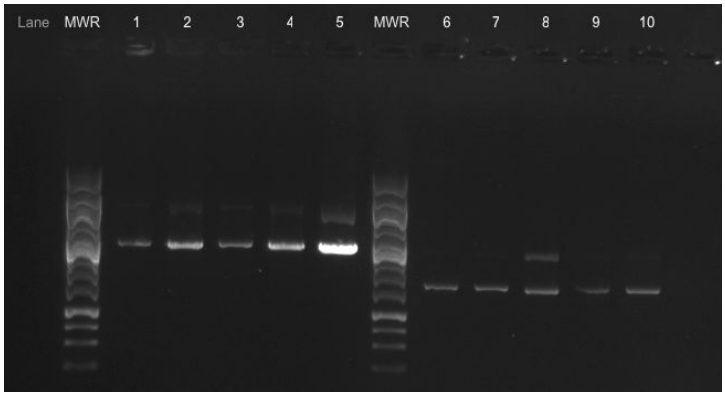
Ann Gel 1% agarose



Reida Gel .7% agarose



Christina Gel .4% agarose



- Gel extraction results were very poor

June 11th 2015

Objectives:

- Inoculate the cultures into a liquid medium overnight for making the competent cells
- Transform rehydrated luciferase plasmids from plate kit

Procedures:

- Made more agar plates

June 12th 2015

Objectives:

- Make competent cells by treating them with CaCl2

Procedures:

- Used the protocol Professor Li Wenshu would normally use to make competent cells

Results:

- We forgot to autoclave a flask to grow the culture in 200mL LB. We had to autoclave the flask first but we did not want to leave the bacteria growing while we were waiting for the

autoclave. Therefore, we transferred 500µl of the the medium inoculated overnight into a new 5mL LB Broth and kept that incubated until the autoclave was done. We did this at about 10:40am. After we got the autoclaved medium, we started the inoculation and ended at about 4:00 pm at OD600: 0.382A.

- We also messed up the glycerol stock solution, instead of doing 50% glycerol we made 33.3% glycerol but this can be overcome by just putting more glycerol than CaCl2.

June 15th 2015

Objectives:

- Perform transformation efficiency on the first batch of competent cells made last week.

Procedure:

- Used standard transformation protocol with several controls and e different recovery times (40 minutes and 2 hours) to see which would yield better results.

Results:

Plate	**Bacteria/Plasmid**	**Recovery Time**	**Observation**
LB + Amp	Commercial DH5 α	40 Minutes	Red Colonies Grew
LB + Amp	Commercial DH5 α	2 Hours	Red Colonies Grew
LB + Amp	New DH5 α	40 Minutes	N/A
LB + Amp	New DH5 α	2 Hours	N/A
LB	Commercial DH5 α	40 Minutes	Bacterial Swab
LB	Commercial DH5 α	2 Hours	Bacterial Swab
LB	New DH5 α	40 Minutes	Colonies Grew
LB	New DH5 α	2 Hours	Small Colonies Grew

- Most optimal for growth: Commercial DH5 α for 40 minutes.
- Competent cells has low to zero competency. Should revise method and do it again (this time autoclave flask beforehand)

June 17th 2015

Objectives:

- Make more competent cells (have innoculated some in SOC broth the day before)
- Do miniprep of transformed plasmids
- Make 15 LB + Amp Plates

Procedures:

- Used the same protocol to make competent cells, but have prepared an autoclaved flask beforehand
- Miniprep using tiangen kit protocol

Results:

- Made 69 tubes of DH5α in total, still need to check the transformation efficiency
- Transformation efficiency results showed that there was little to zero efficiency. What we can do as suggested by Prof Kang is to increase the cell density. Incubate 5µL overnight in 500mL culture. In the final step, only resuspend in 1mL of CaCl2 + Glycerol instead of 50mL
- Miniprep results

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor	Name
1	3F_K880005_1	6/17/2015 14:24:00	43.8	ng/µl	0.877	0.456	1.92	1.06	DNA	50	p/rbs
2	3F_K880005_2	6/17/2015 14:29:00	22	ng/µl	0.44	0.229	1.92	1.9	DNA	50	p/rbs
3	16E_K081005_1	6/17/2015 14:34:00	17.3	ng/µl	0.346	0.189	1.84	0.53	DNA	50	p/rbs2
4	16E_K081005_2	6/17/2015 14:36:00	17.3	ng/µl	0.346	0.185	1.87	0.57	DNA	50	p/rbs2
5	19E_K592009_1	6/17/2015 14:39:00	23.4	ng/µl	0.467	0.226	2.07	1.21	DNA	50	blue chromoprotein
6	19E_K592009_2	6/17/2015 14:40:00	18.5	ng/µl	0.37	0.187	1.98	1.54	DNA	50	blue chromoprotein
7	2I_K592011_1	6/17/2015 14:43:00	23.5	ng/µl	0.469	0.235	2	1.59	DNA	50	green chromoprotein
8	2I_K592011_2	6/17/2015 14:45:00	25.1	ng/µl	0.502	0.255	1.97	1.64	DNA	50	green chromoprotein

9	5F_K1467201_1	6/17/2015 14:47:00	16.8	ng/µl	0.336	0.17	1.98	1.5	DNA	50	gg blue chromoprotein
10	5F_K1467201_2	6/17/2015 14:49:00	19.1	ng/µl	0.382	0.2	1.91	1.46	DNA	50	gg blue chromoprotein
11	6K_K1033910_1	6/17/2015 14:51:00	14.6	ng/µl	0.292	0.152	1.93	1.09	DNA	50	yellow chromoprotein
12	6K_K1033910_2	6/17/2015 14:56:00	8.1	ng/µl	0.163	0.074	2.19	1.35	DNA	50	yellow chromoprotein
13	6M-K1033916-1	6/17/2015 15:13:00	13.6	ng/µl	0.272	0.119	2.28	0.99	DNA	50	lime chromoprotein
14	6M-K1033916-2	6/17/2015 15:15:00	12.7	ng/µl	0.254	0.115	2.2	1.86	DNA	50	lime chromoprotein
15	13L-E0040-1	6/17/2015 15:17:00	17.4	ng/µl	0.347	0.166	2.09	1.61	DNA	50	amp backbone
16	13L-E0040-2	6/17/2015 15:19:00	14	ng/µl	0.28	0.131	2.13	2.17	DNA	50	amp backbone
17	4N-E0020-1	6/17/2015 15:25:00	12.4	ng/µl	0.249	0.117	2.12	1.78	DNA	50	chloram. backbone

June 18th 2015

Objectives:

- Perform restriction digest for the transformed parts using iGEM protocol

Procedures:

- Use iGEM protocol to do restriction digest of p/rbs, p/rbs 2, blue chromoprotein, and linearized backbone so they can be ligated as such:

	Part A	Part B	Backbone
1.	p/rbs	Blue Chromoprotein	Our amplified Backbone
2.	p/rbs	Blue Chromoprotein	Linearized Backbone (iGEM)
3.	p/rbs 2	Blue Chromoprotein	Our amplified Backbone
4.	p/rbs 2	Blue Chromoprotein	Linearized Backbone (iGEM)

- Amounts of enzymes used (and order that they are inserted) - iGEM Protocol:

	Part A	Part B	Backbone
DNA	250ng	250ng	250ng
H2O	Adjust to 16µl	Adjust to 16µl	Adjust to 16µl
NEB Buffer 2.0	2.5 µL	2.5 µL	2.5 µL

BSA	0.5µL	0.5µL	0.5µL
Enzyme 1	0.5µL EcoRI	0.5µL XbaI	0.5µL EcoRI
Enzyme 2	0.5µL SpeI	0.5µL PstI	0.5µL PstI
Enzyme 3			0.5µL DpnI

Total volume should be 20µL. Incubate at 37ºC for 30 minutes, then at 80ºC for 20 minutes using a thermal cycler. Run a gel and use 2µL of the digest for ligations

Results:

- Gel electrophoresis shows that the restriction digest did not work
- Cause: we did not use the protocol from the reagent but instead used the iGEM protocol, we decided to re-do the protocol according to NEB but only cutting the ampicillin backbone (to see if it works with one reagent before doing everything else).

June 19th 2015

Objectives:

- Restriction digest of ampicillin backbone with XbaI and PstI enzyme using NEB and refined iGEM Protocol

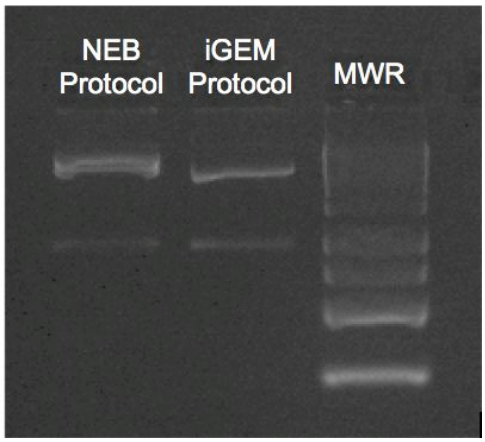
Procedure:

- (NEB Protocol), the iGEM Protocol was done by Spencer

DNA Concentration	57 ng/µL
Amount of DNA	17.54 µL
Xba I	1µL
Pst I	1µL
Buffer 3.1	5µL
Water	25.46µL
Incubation Time	37ºC for 10-15 minutes, 80ºC for 1 Hour

Results:

- Gel electrophoresis results using NEB and refined iGEM Protocol (2% agarose gel):



First Band is 2500B.P and the second band is ~1000bp. The expected bands were 2079bp and 720bp.

June 23rd 2015

Objectives:

- Restriction Digest of E & S restriciton sites using sequential digest
- Plasmids that are going to be digested:
  - p/rbs (70.5ng/µL)
  - Ampicillin Backbone (57ng/µL)
- Using both protocol from iGEM and the NEB protocol
- Gel electrophoresis of restriction digest products
- Ligation (if restriction digest is successful)

Procedures:

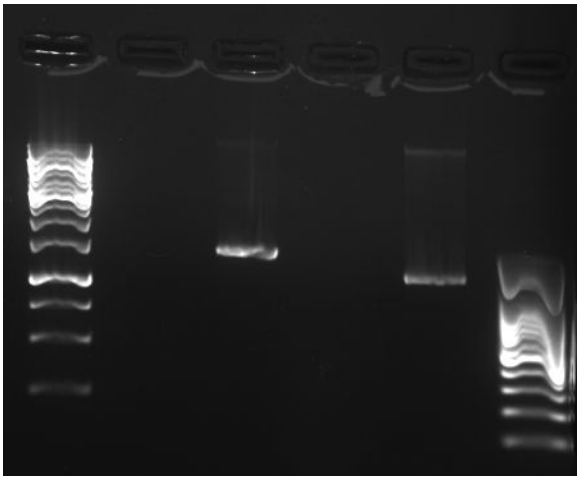
- NEB Protocol as follows:

		p/rbs 2	**Ampicillin Backbone**
	Concentration	70.5 ng/µL	57ng/µL
	Amount of DNA Used	14.18 µL	17.54µL
STEP 1	Cutsmart Buffer	5µL	5µL
	Water	29.82µL	25.45µL
	SpeI	1µL	1µL
	Incubation	37ºC for 5-15 minutes, then 80ºC for 20 minutes	37ºC for 5-15 minutes, then 80ºC for 20 minutes
STEP 2	EcoRI	1µL	1µL
	Buffer 2.1	5µL	5µL

	Water	43-44µL	43-44µL
	Incubation Time	Incubate 37ºC 10-15 minutes, incubate at 65ºC for 20 minutes	Incubate 37ºC 10-15 minutes, incubate at 65ºC for 20 minutes

Results:

- Digest did not work. Gel electrophoresis showed that there were no bands (which means there may be too little DNA). There might be several reasons:
  - We used NEBuffer 2.1 instead of EcoRI Buffer during digestion.
  - Did not put the elution buffer in the center of the column, therefore the sample did not elute h at the last step of sequential digest (elution)
  - Digestion time was not sufficient
- Professor Kang suggested that we need to increase the DNA starting amount (our miniprep samples had very low concentrations)
- Not so sure why our control (the uncut ampicillin backbone and uncut p/rbs) has two bands.



From left to right: 1kb molecular ruler, digested ampicillin backbone, ampicillin plasmid, digested prbs 2, p/rbs 2 plasmid

June 24th 2015

Objectives:

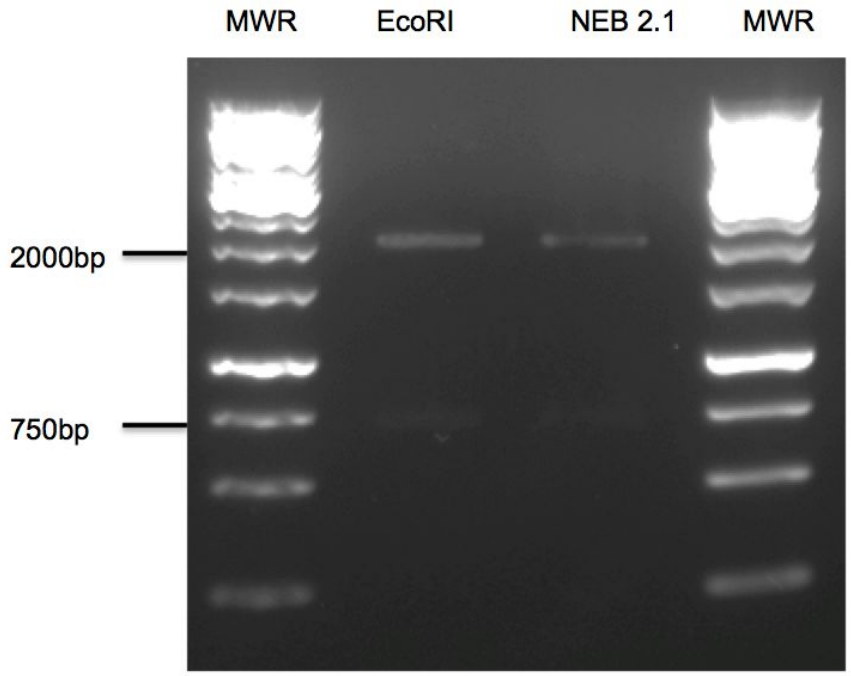
- Make some ampicillin plates
- Prepare for competent cells tomorrow
  - Make 500mL LB Broth
  - In a separate bottle: make 250mL LB Broth
- Do a double digest of Green Chromoprotein (BBa_K592011) with E and S enzyme. (After consulting with Professor Kang we realized that we did not have to do a sequential digest, we just needed to increase the starting amount of DNA)

Procedure:

Concentration	82.3 ng/µL
Volume of DNA Used	1.888µL DNA
EcoRI Enzyme	0.5 µL
SpeI Enzyme	1.0 µL
H2O	20 µL
EcoRI Buffer/NEB Buffer	2 µL
Incubation Time	75 Minutes @ 37ºC and 20 Minutes @ 80ºC

Results:

- The digest was successful as shown in the gel electrophoresis below. We ran two gels: one for the digest using EcoRI buffer and one for the digest using NEB 2.1 buffer - they both seem to exhibit similar results.
- The expected band lengths were: 702bp for the part and 2070bp for the backbone:



Thursday June 25 2015

- **Was not in the lab, Reida did competent cells for me**

Friday June 26th 2015

- Do Restriction Digest for E & P

Procedure:

Concentration	82.3 ng/µL
Volume of DNA Used	1.888µL DNA
EcoRI Enzyme	0.5 µL
PstI Enzyme	0.5 µL
H2O	20 µL
EcoRI Buffer/NEB Buffer	2 µL
Incubation Time	1 Hour @ 37ºC and 20 Minutes @ 80ºC

Results:

- Restriction digest with E & P worked as planned.

June 29 2015

Objectives:

- Do restriction digest, gel electrophoresis, and ligation of these plasmids:
  - Constitutive Promoter + RBS (BBa_K081005)
  - Yellow-Green Chromoprotein (BBa_K1033916)
  - GFP in ampicillin backbone (BBa_E0040)
- Transformation of the ligation products

Procedures

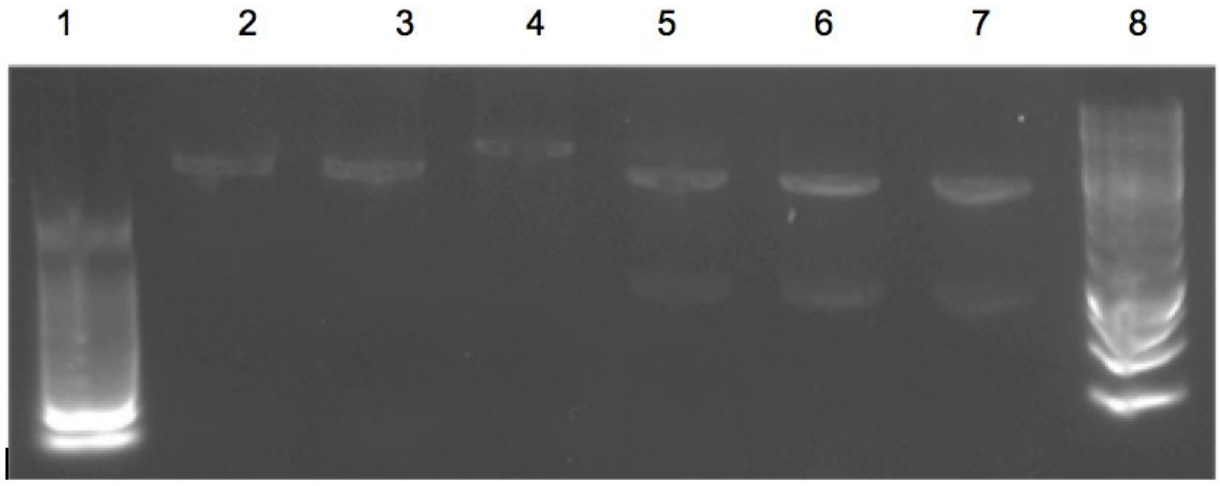
	p/rbs	**yellow/green chromo**	**GFP in amp backbone**
Concentration	153.9 ng/µL	227 ng/µL	106.6 ng/µL
Amount of Plasmid	0.6477µL	0.4387µL	0.9117µL
H2O	7.7523µL	8.8613µL	0.9117µL
Enzyme 1	0.2µL EcoRI	0.2µL XbaI	0.2µL EcoRI
Enzyme 2	0.4µL SpeI	0.2µL PstI	0.2µL PstI
Buffer	1µL EcoRI Buffer/ 1µL NEB 2.1 BUffer	1.µL NEB 3.1	1µL EcoRI 1µL 3.1 NEB Buffer

			1µ 2.1 NEB Buffer
Incubation Time	Incubate at 37ºC for 2 hour, heat kill at 80ºC for 20 minutes		

Results

- In the gel electrophoresis, lanes 2 and 3 (the digested p/rbs) two separate bands did not appear. The same also happened with lane 4, the lane with yellow/green chromoprotein samle. Lanes 5 and 6 *did* show 2 bands, but we can't be sure if they cut correctly because the molecular weight ladder is not legible. We probably used way too little volume of everything in order to see results in the gel electrophoresis.

Lane/Number	Content
1	100bp Ruler
2	p/rbs 2 + EcoRI Buffer
3	p/rbs 2 + NEB 2.1 Buffer
4	Yellow/Green Chromoprotein
5	Backbone + EcoRI Buffer
6	Backbone + NEB 3.1
7	Backbone + NEB 2.1
8	1kb ruler



Gel Electrophoresis Results of Restriction Digest June 29th 2015

- Transformation showed no results. May be due to:
    - First attempt in ligation, need to troubleshoot the process
    - Error carried over from restriction digest (i.e. the parts weren't digested properly, there was not enough DNA, etc)

Tuesday June 30th 2015

Objectives:

- Purifying the backbones (after consultating with the professor, it is best we do gel extraction after digest of the backbones in order to ensure that there is no ligation of the backbone and its original fragment)
    - Restriction Digest of GFP in ampicillin (BBa_E0040) and CFP in chloramphenicol backbone (BBa_E0020)

Procedure:

Sample:	GFP in Amp 1	GFP in Amp 2	CFP in Chlor:
Concentration	80.2ng/µL	160.6ng/µL	180.6ng/µL
Volume of DNA used	25.18µL	18.97µL	11.21µL
H2O	17.82µL	24.04µL	31.79µL
Buffer NEB 2.1	5µL	5µL	5µL
EcoRI	1µL	1µL	1µL
PstI	1µL	1µL	1µL
Incubation Time	37ºC for 1 Hour, Heat kill at 80ºC for 20 Minutes	37ºC for 1 Hour, Heat kill at 80ºC for 20 Minutes	37ºC for 1 Hour, Heat kill at 80ºC for 20 Minutes

July 1st

Objectives:

- Run the samples from yesterday's digestion on a gel
- Gel Extraction of the samples digested yesterday

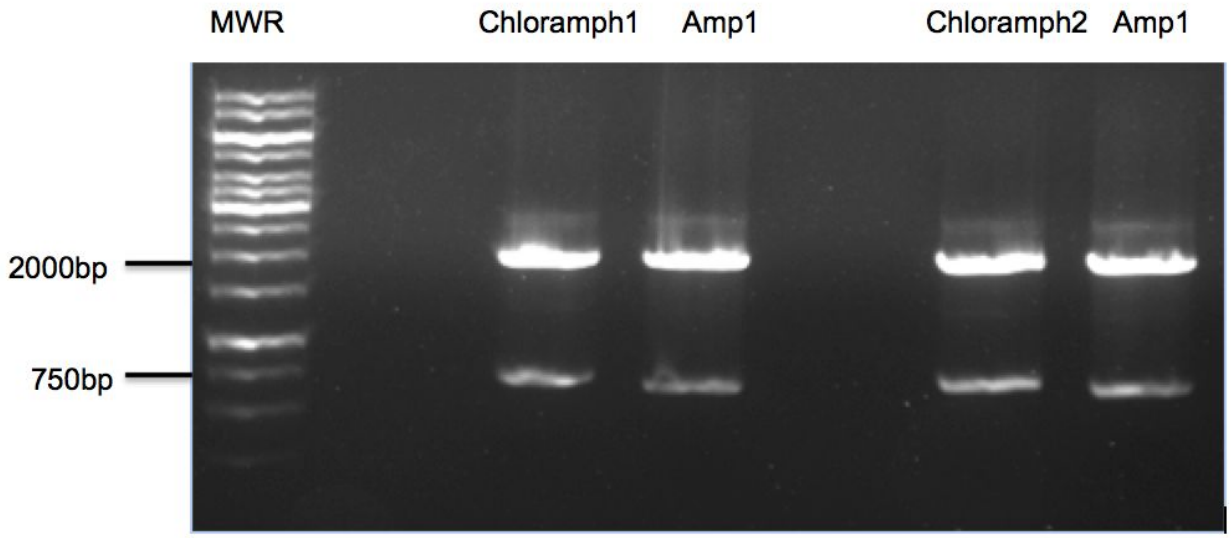
Procedures:

- Gel Electrophoresis using 0.75% gel
- Use TianGen Gel Extraction Kit

Results:

- Gel electrophoresis showed that the digest was indeed successful. (The gel itself was problematic because we forgot to put DuRed and had to submerge the gel in DuRed for >1 Hour)

- The gel extraction results were very low concentration with poor 260/280 results



Gel Electrophoresis of digested GFP in Ampicillin Backbone and CFP in Chloramphenicol backbone

Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
chlor	Student	7/1/2015 17:06:00	27	ng/µl	0.54	0.158	3.41	0.07	DNA	50
chlor	Student	7/1/2015 17:07:00	26.9	ng/µl	0.539	0.175	3.08	0.07	DNA	50
amp	Student	7/1/2015 17:08:00	29.7	ng/µl	0.593	0.215	2.76	0.05	DNA	50

Nanodrop results after Gel Extraction of ampicillin and chloramphenicol backbones

July 2nd

Objectives:

- Do Gel extraction of digested chloramphenicol backbone using the QianGen kit

Procedures:

- Gel Electrophoresis of digested CFP in chloramphenicol backbone
- Gel extraction using QianGen kit

Results:

- Good concentration and 260/280 ratio

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor

1	Chloramphenicol bb #1	Student	7/2/2015 14:53:00	13.7	ng/µl	0.274	0.143	1.91	0.02	DNA	50
2	Chloramphenicol bb #2	Student	7/2/2015 14:37:00	27.8	ng/µl	0.555	0.286	1.94	0.05	DNA	50

Nanodrop results for Gel Extraction of CFP on Chloramphenicol backbone

July 3rd 2015

Objectives:

- Gel extraction of ampicillin backbone
- Ligation of ampicillin backbone (psb1A2), yellow-green chromoprotein, and p/rbs

Procedures:

- Gel electrophoresis, Gel extraction with QianGen kit
- Ligation (using NEB protocol)

Sample/Procedure	**Amount**
0.6925µL	Ampicillin Backbone
1.2766µL	p/Rbs
1.66126µL	Yellow/Green Chromoprotein
1µL	T4 Buffer
0.5µL	Ligase
4.86857 µL	H2O
Incubation:	30 Minutes @ 16ºC, 20 minutes at 80ºC

Results:

- Good yield of ampicillin backbone (psb1A2)

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
	ampicillin	Student	7/3/2015 14:03:00	36.1	ng/µl	0.723	0.385	1.88	0.76	DNA	50

Nanodrop result of gel extraction of GFP in ampicillin backbone

Monday 6 July 2015

Objectives:

- Transformation of Ligation Products

Procedure:

- Transformation Protocol on LB + Amp plate and LB plate (control)

Results:

- (Observed the next day)
  - LB + Amp: No Colonies
  - LB: no Colonies
- Ligation did not work. Did not run gel/have a ligation control to troubleshoot the procedure.
- Next transformation, we should include the following controls:
  - Positive controls: competent cells + known plasmid
  - Negative control: cut backbone + part + ligase

Tuesday July 7th

(Was not in Lab)

Wednesday July 8th

Objectives

- Miniprep inoculated luciferase & do gel electrophoresis
- Innoculate the ligation samples Reida transformed yesterday

Procedure

- Miniprep with Biomiga
- Inoculation of ligation samples (in SOC broth)

Results:

- Miniprep Results:

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	2I RFP 1	Student	7/8/2015 14:37:00	115.7	ng/µl	2.314	1.169	1.98	2.25	DNA	50
2	2I RFP 2	Student	7/8/2015 14:39:00	137.8	ng/µl	2.756	1.426	1.93	2.18	DNA	50
3	3J Renilla	Student	7/8/2015 14:39:00	74	ng/µl	1.48	0.763	1.94	1.81	DNA	50
4	2H Apple Fragrance	Student	7/8/2015 14:40:00	77.5	ng/µl	1.551	0.802	1.93	2.11	DNA	50
5	4J Orange Luc	Student	7/8/2015 14:41:00	139.7	ng/µl	2.794	1.429	1.96	2.21	DNA	50
6	2F Epic Luc	Student	7/8/2015 14:42:00	56.3	ng/µl	1.127	0.58	1.94	2.04	DNA	50
7	2H Green Luc	Student	7/8/2015 14:43:00	79.6	ng/µl	1.591	0.833	1.91	2.1	DNA	50
8	2J Yellow Luc	Student	7/8/2015 14:44:00	79.6	ng/µl	1.592	0.81	1.97	2.13	DNA	50

9	4L Lux Operon	Student	7/8/2015 14:44:00	77.5	ng/µl	1.549	0.809	1.92	2.17	DNA	50
10	2D Red Luc	Student	7/8/2015 14:45:00	261.7	ng/µl	5.233	2.654	1.97	2.26	DNA	50

- Gel electrophoresis results were not as expected.

Thursday July 9th 2015

Objectives:

- Miniprep the ligation products (inoculated overnight)
- Restriction Digest of the mini-prepped ligation products
- Transform luciferase (mini prepped yesterday)

Procedures:

- Use Biomiga protocol to miniprep the ligation products
- Standard transformation procedure
- Restriction digest using miniprep sample #4 (146.7ng/µL), E & S enzymes. Following NEB protocol

Results

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	EcoRI 1	Student	7/9/2015 13:10:00	145.3	ng/µl	2.905	1.399	2.08	2.25	DNA	50
2	EcoRI 2	Student	7/9/2015 13:11:00	102.8	ng/µl	2.056	1.007	2.04	2.18	DNA	50
3	EcoRI 3	Student	7/9/2015 13:12:00	68.5	ng/µl	1.371	0.7	1.96	1.98	DNA	50
4	Buffer 2.1 1	Student	7/9/2015 13:13:00	146.7	ng/µl	2.935	1.423	2.06	2.16	DNA	50
5	Buffer 2.1 2	Student	7/9/2015 13:13:00	113.6	ng/µl	2.272	1.109	2.05	2.13	DNA	50
6	Buffer 2.1 3	Student	7/9/2015 13:14:00	210.5	ng/µl	4.209	2.009	2.1	2.28	DNA	50

Nanodrop results of p/rbs + yellow/green chromoprotein digested with buffers NEB 2.1 and EcoRI

- Transformation results (viewed the next day)
  - All grew except YFP

Monday, July 13th 2015

Objectives:

- Gel electrophoresis & extraction of backbones

Procedures:

- Gel electrophoresis & Gel extraction using QianGel kit

Results:

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	Chloramphenicol Backbone	Student	7/13/2015 15:48:00	37.3	ng/µl	0.747	0.388	1.93	0.05	DNA	50
2	Ampicillin backbone	Student	7/13/2015 15:53:00	31	ng/µl	0.621	0.365	1.7	0.04	DNA	50

Tuesday, July 14th 2015

Objectives:

- Take an inventory of all plates
- Innoculate cultures of p/rbs + chromoprotein
- Transform YFP

Wednesday, July 15th 2015

Objectives

- Make SOC culture, make small LB + chloramphenicol plates
- Miniprep inoculated cultures:
  - p/rbs + green chromoprotein
  - p/rbs + yellow chromoprotein
  - p/rbs + blue chromoprotein
  - p/rbs + lime chromoprotein
  - Final product (p/rbs + lime + terminator)
  - p/rbs + yellow green chromoprotein
  - mcherry
  - GFP
  - RFP
- Re-innoculate the plasmids that had poor miniprep results:
  - Final plasmid 1 & 2
  - p/rbs + Green 1 & 2

Procedure

- Miniprep using Biomiga miniprep kit

Results

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	m.cherry 1	Student	7/15/2015 14:26:00	60.5	ng/µl	1.211	0.632	1.92	1.78	DNA	50
2	m.cherry 2	Student	7/15/2015 14:28:00	113.9	ng/µl	2.278	1.165	1.96	2.32	DNA	50
3	rfp 1	Student	7/15/2015 14:29:00	218.1	ng/µl	4.362	2.145	2.03	2.27	DNA	50
4	rfp 2	Student	7/15/2015 14:30:00	258.4	ng/µl	5.168	2.544	2.03	2.13	DNA	50
5	gfp 1	Student	7/15/2015 14:31:00	144.4	ng/µl	2.889	1.477	1.96	2.25	DNA	50
6	gfp 2	Student	7/15/2015 14:32:00	125.2	ng/µl	2.503	1.264	1.98	2.22	DNA	50
7	p/rbs + green 1	Student	7/15/2015 14:38:00	4.9	ng/µl	0.098	0.07	1.41	0.58	DNA	50
8	p/rbs + green 2	Student	7/15/2015 14:39:00	7.4	ng/µl	0.147	0.143	1.03	0.83	DNA	50
9	p/rbs + yellow 1	Student	7/15/2015 14:41:00	87.6	ng/µl	1.752	0.926	1.89	2.22	DNA	50
10	p/rbs + yellow 2	Student	7/15/2015 14:42:00	3	ng/µl	0.06	0.036	1.67	1.39	DNA	50
11	p/rbs + ggblue 1	Student	7/15/2015 14:43:00	71.3	ng/µl	1.426	0.755	1.89	2.03	DNA	50
13	p/rbs + ggblue 2(Reblank)	Student	7/15/2015 15:25:00	21.5	ng/µl	0.431	0.222	1.94	1.35	DNA	50
14	p/rbs + ggblue 11	Student	7/15/2015 15:26:00	2.4	ng/µl	0.048	0.013	3.72	0.53	DNA	50
15	p/rbs + ggblue 12	Student	7/15/2015 15:27:00	19.6	ng/µl	0.392	0.197	1.99	0.83	DNA	50
16	p/rbs + yellow/green	Student	7/15/2015 15:28:00	140.8	ng/µl	2.817	1.418	1.99	2.08	DNA	50

Nanodrop results of July 15th's Miniprep. Those highlighted in red were thrown away because of poor results.

Thursday July 16th 2015

Objectives:

- Miniprep innoculated cultures:
    - Final plasmid 1 & 2
    - p.rbs + green 1 & 2
- Restriction digest & ligation of:
    - p/rbs + yellow and terminator
    - p/rbs + gg blue 1 and terminator
    - p/rbs + yellow/green chromoprotein
- Transformation of ligated products from today
- Transform some RFP in different arrangements
- Reinoculation of p/rbs and various chromoproteins:
    - gg blue chromoprotein (K1467201)
    - blue green chromoprotein (K592011)
    - blue chromoprotein (K592009)
    - yellow chromoprotein (K1033910)
    - yellow-green chromoprotein (K1033916)

Procedures:

- Restriction digest follows NEB Protocol, but utilizes formula below to find minimum amount of DNA that needs to be used if a gel was run:

$$V\ of\ DNA\ = \frac{Total\ Digest\ Volume\ (50mL) \times Weight\ of\ DNA\ (20ng)}{(Initial\ Concentration\ of\ DNA)\ (\frac{Part\ Size}{Total\ Plasmid})(Gel\ Loading\ Volume)}$$

	Yellow	**GG Blue 1**	**Yellow/Green**
Concentration	87.6ng/µL	71.3ng/µL	140ng/µL
DNA Volume	11.41µL	14.03µL	7.1µL
H2O	31.59µL	28.97µL	35.9µL
EcoRI	1µL	1µL	1µL
SpeI	1µL	1µL	1µL
Buffer	5µL	5µL	5µL
Incubation	Incubate at 37ºC for 1 Hour, Heatkill at 80ºC for 20 Minutes		

- Ligation was also according to NEB Protocol but utilizes the formula below to find the minimum amount of DNA that needs to be used for an efficient ligation:

$$V\ of\ Insert\ DNA = \frac{(25ng)\ (Molar\ Ratio\ of\ Insert/Vector)}{[Initial\ DNA\ Concentration](\frac{DNA\ Volume}{Total\ Volume})(\frac{Part\ Size}{Total\ Plasmid\ Size})}$$

	Yellow Chromoprotein	**GG Blue Chromoprotein**	**Yellow-green Chromoprotein**

p/rbs + chromoprotein	2.75µL	2.75µL	2.75µL
Terminator	2µL	2µL	2µL
Backbone	2µL	2µL	2µL
T4 Ligase Buffer	1µL	1µL	1µL
Ligase Enzyme	0.5µL	0.5µL	0.5µL
Water	1.75µL	1.75µL	1.75µL

Results:

- (Observed the next day)
  - p/rbs + yellow + terminator: growth, no color
  - p/rbs + gg blue + terminator: growth, no color
  - p/rbs + yellow/green + terminator: no color

Friday July 17th 2015

Objectives:

- Miniprep samples from yesterday + backbone

Procedures:

- Miniprep with Biomiga protocol

Results:

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	p/rbs + lime 1	Student	7/17/2015 13:16:00	153.5	ng/µl	3.069	1.547	1.98	1.78	DNA	50
2	p/rbs + lime 2	Student	7/17/2015 13:17:00	227.2	ng/µl	4.543	2.2	2.06	2.16	DNA	50
3	p/rbs + green 1	Student	7/17/2015 13:18:00	66.9	ng/µl	1.338	0.666	2.01	1.93	DNA	50
4	p/rbs + green 2	Student	7/17/2015 13:19:00	103.5	ng/µl	2.07	1.002	2.07	2.11	DNA	50
5	amp backbone	Student	7/17/2015 13:20:00	78.9	ng/µl	1.579	0.832	1.9	1.64	DNA	50

6	chloramphenicol backbone	Student	7/17/2015 13:21:00	113.7	ng/µl	2.274	1.182	1.92	1.89	DNA	50
7	p/rbs	Student	7/17/2015 13:22:00	225.8	ng/µl	4.516	2.386	1.89	2.08	DNA	50
8	p/rbs + yellow NEB 2.1	Student	7/17/2015 13:24:00	88	ng/µl	1.76	0.885	1.99	1.99	DNA	50
9	p/rbs + yellow EcoRI	Student	7/17/2015 13:25:00	93.9	ng/µl	1.878	0.954	1.97	1.95	DNA	50
10	p/rbs + blue NEB 2.1	Student	7/17/2015 13:26:00	89.9	ng/µl	1.799	0.906	1.98	1.86	DNA	50
11	p/rbs + blue EcoRI	Student	7/17/2015 13:27:00	72.9	ng/µl	1.459	0.73	2	1.93	DNA	50
12	p/rbs + ggblue NEB 2.1	Student	7/17/2015 13:28:00	114.3	ng/µl	2.286	1.083	2.11	2.01	DNA	50
13	p/rbs + ggblue EcoRI	Student	7/17/2015 13:30:00	89	ng/µl	1.781	0.885	2.01	1.99	DNA	50

Nanodrop results from miniprep on 17 July 2015

Tuesday, July 28th 2015

Objectives:

- Transform luciferase, chromoproteins, fluorescent proteins, promoters, terminators, and backbones from the original iGem kit (some would require re-hydrating)

Procedures:

- Standard transformation protocol. However, we recovered for only 1 hour instead of 2, and used 20µL DH5α instead of 50µL.

Results:

Kit Plate Number	Plasmid Location	Code	Backbone	Name	Result (X = Did not Transform)
3752	4L	BBa_K325909	psb1C3	lux under pBad	X
3752	2D	BBa_K324219	psb1C3	red firefly	X
3752	2F	BBa_K325108	psb1C3	epic firefly	X
3752	4J	BBa_K325218	psb1C3	orange firefly	X
3752	2H	BBa_K325209	psb1C3	green firefly	X
3752	2J	BBa_K325259	psb1C3	yellow firefly	

3755	6K	BBa_K1033910	psb1C3	fwYellow yellow chromoprotein	X
3755	6M	BBa_K1033916	psb1C3	Lime yellow-green chromoprotein	X
3752	19E	BBa_K592009	psb1C3	amilCP blue chromoprotein	
3755	2I	BBa_K592011	psb1C3	CJBlue green chromoprotein	X
3756	5F	BBa_K1467201	psb1C3	gg amilCP blue chromoprotein	X
3755	4B	BBa_J04450	psb1C3	RFP Coding Device	
3755	24D	BBa_E0840	psb1C3	GFP	X
3756	14D	BBa_K1429001	psb1C3	RFP	
3756	14F	BBa_K1429002	psb1C3	CFP	
3753	3F	BBa_K880005	psb1C3	RBS promoter	
3754	16E	BBa_K081005	psb1C3	const. promoter	X
3755	2H	BBa_J04450	psb1A3	RFP on AMP	X
3752	24P	BBa_J04450	psb1C3	RFP on Chlo	X
3754	3F	BBa_B0015	psb1C3	Terminator	
3754	6C	BBa_B0017	psb1C3	Terminator	

- The small amount of e.coli we used coupled with the reduced recovery time might have contributed to the poor transformation results.

Wednesday July 29th 2015

Objectives:

- Digest blue chromoprotein
- Transformation of parts that did not work
- Inoculation of the parts that transformed successfully from July 28th

Procedures:

- NEB Protocol
- Gel electrophoresis to see results

Results:

- Gel electrophoresis showed that digest worked and the part could be ligated tomorrow.

Thursday July 30th 2015

Objectives

- Ligation of yesterday's digested product (blue chromoprotein to p/rbs)
- Gel extraction of ampicillin backbone and chloramphenicol backbone

- Inoculation of the parts transformed yesterday

Procedure:

July 28th 2015

Objectives:

- Transform luciferase, chromoproteins, fluorescent proteins, promoters, terminators, and backbones
- Make more LB and chloramphenicol plates

Procedures:

- Standard transform protocol was used with an incubation time of one hour, 20 uL of DH5a and 2ul of plasmid were used per transformation

Results:

- Transformations were successful for:
  - Luciferase: 2J - BBa_K325259
  - Chromoprotein: 19E - BBa_K592009
  - Fluorescent protein: 4B - BBa_J04450; 14D - BBa_K1429001; 14F - BBa_ K1429002
  - Promoters/Terminators/Backbones: 3F - BBa_K880005; 3F - BBa_B0015; 6C - BBa_B0017
- Transformations unsuccessful for:
  - Luciferase: 4L - BBa_K325909; 2D - BBa_K325219; 2F - BBa_K325108; 4J - BBa_K325218; 2H - BBa_K325209
  - Chromoprotein: 6K - BBa_K1033910; 6M - BBa_K1033916; 2I - BBa_K592011; 5F - BBa_K1467201
  - Fluorescent Protein: 24D - BBa_E0840
  - Promoters/Terminators/Backbones: 16E - BBa_K081005; 2H - BBa_J04450; 24P - BBa_J04450
- Transformations were likely unsuccessful because plasmids were not completely mixed with DH5a

July 29th 2015

Objectives:

- Inoculate successfully transformed parts from July 28th (2J, 19E, 4B, 14D, 14F, 3F - BBa_K880005; 3F - BBa_B0015, 6C)
- Retransform unsuccessfully transformed parts from July 28th (4L, 2D, 2F, 4J, 2H, 6K, 6M, 2I, 5F, 24D, 16E, 2H, 24P)
- Restriction digest for blue chromoprotein, yellow chromoprotein, and lime chromoprotein sequenced by IDT and chloramphenicol backbone

Procedures:

- Standard inoculation procedure was used
- Standard transformation protocol was used
- 3A assembly restriction digest for chloramphenicol and ampicillin backbones

Results:

- Successful transformation:
  - 16E; 5F; 6K; 24D; 2H - BBa_J04450
- Unsuccessful transformation:
  - 2I, 6M, 2F, 4J, 4L, 2H, 24P, 2D
- Transformation were likely unsuccessful because not enough plasmid was available for transformation (<0.5 ul)
- Restriction digest was unsuccessful because the gel was run in the wrong direction

July 30th 2015

Objectives:

- Make more chloramphenicol/chloramphenicol + arabinose plates
- Inoculation was done for the successful transformation from July 29th (parts: 16E, 5F, 6K, 24D, 2H)
- Miniprep inoculated bacteria from July 29th (2J, 19E, 4B, 14D, 14F, 3F - BBa_K880005; 3F - BBa_B0015, 6C)

Procedures:

- Biomiga Plasmid Miniprep Kit and protocol was used
-

Results:

-

July 31st 2015

Objectives:

- Miniprep inoculated plates from July 30th (plates 16E, 5F, 6K, 24D, 2H)
- Gel extraction for chloramphenicol and ampicillin backbones

Procedures:

- Biomiga Plasmid Miniprep Kit and protocol was used
- Qiagen Gel Extraction Kit and protocol was used

Results:

Miniprep

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	24D GFP terminator 1	Student	7/31/2015 12:02:00	249.2	ng/µl	4.984	2.372	2.1	2.34	DNA	50
3	24D GFP terminator 2	Student	7/31/2015 12:04:00	768.7	ng/µl	15.375	7.219	2.13	2.33	DNA	50
4	5F GGBlue Chromoprotein	Student	7/31/2015	157.9	ng/µl	3.158	1.554	2.03	2.2	DNA	50

			12:05:00								
5	5F GGBlue Chromoprotein 2	Student	7/31/2015 12:06:00	330.5	ng/µl	6.611	3.157	2.09	2.26	DNA	50
6	6K fwyellow 1	Student	7/31/2015 12:07:00	137.3	ng/µl	2.746	1.423	1.93	2.02	DNA	50
7	16E const promoter 1	Student	7/31/2015 12:08:00	348	ng/µl	6.959	3.308	2.1	2.23	DNA	50
8	16E const promoter 2	Student	7/31/2015 12:09:00	425.6	ng/µl	8.512	4.019	2.12	2.29	DNA	50

Gel Extraction

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	ampicillin backbone	Student	7/31/2015 14:40:00	18	ng/µl	0.36	0.186	1.94	0.03	DNA	50
2	chloramphenicol backbone	Student	7/31/2015 14:41:00	9.5	ng/µl	0.19	0.102	1.87	0.05	DNA	50

- Gel Extraction results were unsatisfactory; low concentration

August 3rd 2015

Objectives:

- Resuspension of luciferase plasmid from fudan plate kits
- Inoculation of yellow luciferase in 5 different arabinose concentrations
- Inoculation of chloramphenicol and Ampicillin Backbone

Procedures:

- Looked at the Cambridge team in how they characterized the luciferase based on different arabinose concentrations (http://2010.igem.org/Team:Cambridge/Bioluminescence/Vibrio_Characterisation)
- Decidedto do the concentrations below:

Arabinose Concentration (in M)	**Arabinose Concentration in mg/ml**

0.003M	0.45039mg/mL
0.0045M	0.6756mg/mL
0.006M	9.0078mg/mL
0.0075M	11.25975mg/mL
0.01M	1.5013mg/mL

- Made arabinose stock solution of 100mg/mL (1g in 10mL), altered the volumes
- Inoculated overnight

Results:

- **(Observed the next day)** No fluorescence in all of the tubes